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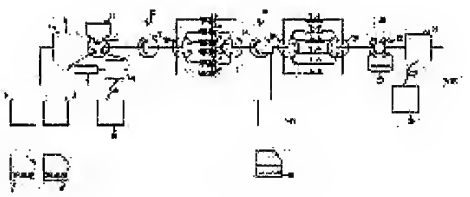
(54) LIQUID CHROMATOGRAPHY

(57)Abstract:

PURPOSE: To allow complicated separation of a multicomponent system, e.g. optical resolution of amino acids, by injecting a sample only once.

CONSTITUTION: In a liquid chromatography where first and second columns are coupled, a plurality of empty columns 15 and a plurality of concentrated columns 21 are coupled between the first and second columns 7, 23. A sample S is fed to the first column 7 using a first eluate composed of a mixture solvent of a rich solvent 2 and a first lean solvent 4 thus separating the components roughly. Each fraction eluted from the first column 7 is sampled by each empty column 15 and then the fraction is eluted from each empty column 15 by means of the first eluate. Subsequently, a second lean solvent 4 is added thereto before it is introduced to each concentrated column 21. The components of each fraction are then captured by respective concentrated columns 21 and subsequently eluted using a second eluate 18 before it is fed to the second column 23 to be separated.



* NOTICES *

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application]This invention relates to the suitable liquid chromatography for automation of high separation and refining of a multicomponent system, such as DL division of amino acid, and analysis especially about the separation method of an ingredient based on liquid chromatography, and its device.
[0002]
[Description of the Prior Art]When liquid chromatography analyzes the mixture of the substance with which a complicated substance or structure was similar, in a single isolation column, each ingredient in a sample cannot be separated in many cases. In this case, the kind of an isolation column, an eluate, or its both must be changed, and multiple-times analysis must be conducted. However, many devices and much analytical time are needed for conducting multiple-times analysis. Since multiple-times pouring of the sample is carried out, a precious sample is wasted in many cases. It is possible to connect the 1st column and the 2nd column with which separation modes differ mutually these days, and to separate an ingredient, The technique using the liquid chromatography which connected the GPC column separated with a molecular weight and the opposite phase column separated with polarity is proposed (JP,61-140861,A and JP,2-171653,A each gazette).
[0003]By the system of the liquid chromatography which considered opposite phase column connection as the GPC column, the back pressure of an opposite phase column is applied to a GPC column, and it is easy to damage GPC column packing material of a weak structure to a pressure because of that of three-dimensional polymerization polymer. Therefore, the linkage method of the GPC column through a mono tube or a valve and an opposite phase column is directly impossible practical, A trap or an enriching column is made to intervene in the middle of the 1st column and the 2nd column in consideration of the problem of the above-mentioned

back pressure actually, After carrying out the trap of the eluate from the 1st column to a trap pipe, water is added to the trap liquid and it introduces into an enriching column, and an objective component is condensed, the 2nd column analyzes, and the method of acquiring a good peak is reported.

[0004]However, in this passage constitution, it condensed with the enriching column from the null loop, the passage direction of the eluate was made reverse, and the ingredient is introduced into the 2nd column. In this case, since separation modes differ in the combination of a GPC column and an opposite phase column, there is no trouble like ** in separation, but. For example, when the 1st column and the 2nd column analyze an amino acid derivative using an opposite phase ODS system column, where rough separation of DL object in the 1st column is maintained, condense an enriching column, but. the time of introducing an ingredient to the 2nd column -- a passage constitution top eluate -- concentration operation and an opposite direction -- not passing -- separation efficiency is bad, in order not to obtain, but for the peak rough-separated in the enriching column to unify again and for the 2nd column to separate anew. Since there is only one trap pipe, when there are two or more peak groups which need reanalyses, whenever the peak group to wish is eluted, a main pump is stopped, the 2nd column analyzes, or a sample will be poured in each time, the target peak will be analyzed separately, and analytical practice becomes very complicated.

[0005]

[Problem(s) to be Solved by the Invention]This invention solves this problem, in a single analysis condition, it enables complicated multicomponent separation to which a non-detachable structure was similar, and an object of this invention is to provide the liquid chromatography with which various separation results are once obtained by pouring.

[0006]

[Means for Solving the Problem and its Function]In order that this invention persons may solve the above-mentioned problem, as a result of repeating examination wholeheartedly, liquid chromatography which connected two or more vacuum column and two or more enriching columns, and specified those operations between the 1st column and the 2nd column found out attaining the above-mentioned purpose, and reached this invention.

[0007]Namely, in liquid chromatography with which this invention connected the 1st column and the 2nd column, Two or more vacuum column (15) and two or more enriching columns (21) are connected between the 1st column (7) and the 2nd column (23), Feed a sample (S) into the 1st column (7) using the 1st eluate that consists of a mixed solvent of a good solvent (2) and the 1st poor solvent (4), and an ingredient is rough-separated, Extract each fraction eluted from the 1st column (7) to each vacuum column (15),; Rank second, and a fraction of each vacuum column (15) is made to flow out by the 1st eluate, Add the 2nd poor solvent (18) to this, and it introduces into each enriching column (21), After making an ingredient in each

fraction catch in each enriching column (21), It is liquid chromatography making an ingredient in an enriching column (21) eluted using the 2nd eluate, and feeding and separating into the 2nd column (23), It is the way are liquid chromatography which is an opposite phase distribution type column, and also the 1st column (7) and the 2nd column (23) carry out optical resolution of the amino acid especially using it.

[0008]The above is high separation of a sample component to which a mixture of a complicated substance or structure was similar by pouring of once of a sample, a separation method which can carry out high precision analysis certainly and automatically, and its system, and has the following features. ** Passage constitution is one way, and when what kind of column etc. are used, and introducing an ingredient from an enriching column to the 2nd column, there is no aggravation of separation efficiency by inversion of a channel. ** Since it has two or more vacuum column and two or more enriching columns, re-separation of two or more ingredients can be efficiently performed by one analysis.

[0009]

[Example]An example of the multi-column high-speed liquid chromatograph used for operation of the liquid chromatography of this invention is shown in drawing 1. Based on drawing 1, this invention is explained below. The perfusion pump 3 which pours in the good solvent 2, and the perfusion pump 5 which pours in the 1st poor solvent 4 are connected with the inlet 1 of the sample S. As a good solvent, a hydrophilic organic solvent is used and, generally methanol, ethanol, propanol, or acetonitrile is mentioned as the example.

[0010]As the 1st poor solvent, solution is used, for example, the solution of the salt of acid, such as inorganic acid, such as organic acid, such as succinic acid, oxalic acid, and acetic acid, or phosphoric acid, or it, is mentioned. As a cation constituent which forms a salt, lithium, sodium, potassium, ammonium, etc. are preferred. the pH of solution has desirable neutrality -- general -- 4-9.5 -- it is the range of 5-7.5 preferably. A little clathrates, such as crown ether or dextrin, may be added if needed.

[0011]A good solvent and the 1st poor solvent are mixed in the sample inlet 1, the 1st eluate is formed, and the eluate by which the sample S was added is sent to the changeover valve 6. Subsequently, it is sent to the 1st column 7, the detector 8, and the 2nd changeover valve 9 via the changeover valve 6. The recorder 10 displays and records the output of the detector 8, and when the 2nd column 23 separates a sample, the by-path pipe 11 for avoiding the failure of pressure by the 1st column 7 is switched with the changeover valve 6.

[0012]The 1st column 7 is filled up with the bulking agent for separating each ingredient in a sample. As a bulking agent, for example Silica, styrene divinylbenzene copolymer, The silica powder which carried out the surface treatment with the silicon compound which powder, such as polyvinyl alcohol or a polyvinyl pyrrolidone, is used, and has especially **Si-OR (R expresses alkyl group of C_1, C_2, C_8, or C_{18} among formula) basis is preferred. The fraction of

extraction needlessness is discharged from the outlet 12 of an eluate. With the 3rd changeover valve 13 and 4th changeover valve 14, a required fraction is fed into either of two or more vacuum columns 15, and is extracted by the column 15. Operation of the changeover valves 13 and 14 for extracting a required fraction is performed with reference to the display of the recorder 10. A required fraction is extracted by the vacuum column 15-1 - 15-n, respectively, and ranks second, and if the ingredient in the 1st column finishes being eluted, separation with the 2nd column will be performed.

[0013]In order to perform separation with the 2nd column 23, it mixes with the 2nd poor solvent 18 that sends the fraction in the vacuum column 15-1 to the 5th changeover valve 19 by the 1st eluate, and is sent from the 2nd poor solvent perfusion pump 17, and introduces into the predetermined enriching column 21. The enriching column 21-1 - 21-n are filled up with silica or a glass bead as support of a sample component.

The sample component in the fraction with which the 2nd poor solvent was mixed deposits on the support in the enriching column 21.

After the work which makes each fraction extracted by the vacuum column 15 hold to each enriching column 21 is completed, Addition of the 2nd poor solvent 18 is stopped, the 2nd eluate that consists of mixed eluent of the good solvent 2 or the good solvent 2, and the 1st poor solvent 4 is supplied to the predetermined enriching column 21, it is eluted and the ingredient which deposited is fed into the 2nd column 23 via the 6th changeover valve 20 and 7th changeover valve 22.

[0014]In order that the 2nd column 23 may separate each fraction of a sample precisely, it has the same bulking agent as the 1st column 7, but as for the bulking agent of the 2nd column 23, it is preferred to combine so that separability may become heterogeneous [the bulking agent of the 1st column 7]. The sample component separated in the 2nd column 23 is detected with the detector 24, and is discharged from the outlet 25. The recorder 26 carries out indicating record of the output of the detector 24.

[0015]As mentioned above, the main point of operation of this invention is as follows. The introduction valve channel is connected to the 1st column 7-detector 8-outlet 12, a sample is introduced in the state, and the 1st column performs the 1st step of separation. Each fraction separated in this 1st column switches a channel, considers it as the 1st column 7-detector 8-vacuum column 15-outlet 16, and performs the trap of a separation objective component. The obtained rough separation ingredient switches the valves 13 and 14, and a trap is possible for it from 1 to n group. Although the valve channel after the end of separation is switched in the 1st column, it is considered as the by-path pipe 11-vacuum column 15(from 1 to n)-enriching column 21(1-6)-by-path pipe 27-outlet 25 and the ingredient which carried out the trap is certainly condensed to the enriching column 21, By adding the 2nd poor solvent from the pump 17 at this time, the recovery rate of a separation objective component improves further. A valve

channel can be used as the by-path pipe 11-vacuum column 15-enriching column 21-2nd column 23-detector 24 after that, an analysis target ingredient can be re-separated one by one, and this can separate and analyze the non-detachable ingredient conventionally.

[0016]<Example of an experiment> Using an opposite phase column, optical resolution of a small amount of multicomponent amino acid is carried out with the device of this invention, and a single analysis condition explains concretely conventionally the effect of this invention which enabled separation of the non-detachable ingredient.

[0017]A. The 1st column, such as conditions of a device 7: Opposite phase ODS (6 mm[in diameter] x200 mm in length)

Enriching column 15: Opposite phase ODS (6 mm[in diameter] x length 50 mm)

The 2nd column 23: Opposite phase ODS (6 mm[in diameter] x300 mm in length)

Vacuum column 21: 0.5 mm in inside diameter, capacity eluate of 5 ml : Pump 3 Methanol pump 5 0.01M sodium acetate solution (pH=7.0)

Detector : fluorescence detector Excited wavelengths 233-nm fluorescence wavelength 455-nm column temperature : 20 ** [0018]Sample preparation was performed as follows using the pretreatment function of the automatic sampler attached to this device. ** 200microl Add amino acid solution to an empty vial. ** 600microl Add 0.1N sodium-borate solution continuously. ** The 100-ml methanol solution of 0.8g of alt.phthalaldehyde and N-acetyl-L-cysteine was 400microl Added continuously, it was neglected for 2 minutes after 5 times mixing, and the fluorescent derivative of amino acid was prepared.

[0019]B. 1st column 7 5micro of samples l prepared by the separation above-mentioned A which accepts and comes out are poured into the high-speed liquid chromatograph system of drawing 1 which is attached in a fluorescence detector, only the 1st column 7 analyzes, and the result is shown in drawing 2.

[0020]The amino acid which carried out optical resolution, and the amino acid (threonine Thr, histidine His, phenylalanine Phe and ricin Lys, and leucine Leu) which was not divided are seen so that drawing 2 may show. Then, the unattained separation amino acid peak was introduced into preparative isolation, concentration, and the 2nd column 23 at the vacuum column, and re-separation was performed. The following paragraph C explains a result.

[0021]C. The separation C-1. rough separating operation (1) valve in the whole system is operated, and let a channel be the 1st column 7-detector 8-outlet 12.

(2) Make methanol and sodium acetate solution flow from the pumps 3 and 5, respectively.

(3) Inject into the sample inlet 1 the amino acid derivatized in the autosampler.

(4) Dissociate after sample pouring on the gradient conditions which set the eluate as Table 1.

[0022]

[Table 1]

表1

時間（分）	酢酸ナトリウム水溶液（%）	メタノール（%）
0．01	100	0
60．0	40	60

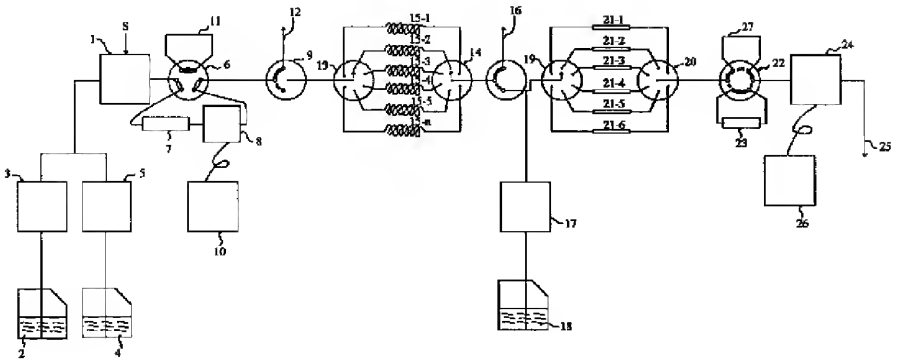
[0023](5) If the peak tip where the detector 8 is insufficient in division is checked, change PARUBU 9 will be operated, switch a channel with the 1st column 7-detector 8-vacuum column 15-1-outlet 16, introduce a peak group insufficient in division to the vacuum column 15-1, and isolate preparatively to it.
(6) If the peak end where the detector 8 is insufficient in separation is checked, the changeover valves 13 and 14 will be operated, and switch a channel to the 1st column 7-detector 8-outlet 12.
(7) If the peak group which still needs re-separation appears, a channel will be switched with the 1st column 7-detector 8-vacuum column 15-2, and it will isolate preparatively.
(8) Subsequent operations repeat operation of (4) to (6) except passing a vacuum column number around.
[0024]C-2. Let a concentration operation (1) valve channel be the by-path pipe 11-vacuum column 15-1-enriching column 21-1-by-path pipe 27-detector 24-outlet 25.
(2) Flow a good solvent and a poor solvent from the pumps 3 and 5, make the poor solvent 18 flow respectively from the pump 17, and carry out the concentration deposit of the preparative isolation fraction in the vacuum column 15-1 to the enriching column 21-1.
(3) Subsequent operations repeat operation of (1) and (2) except passing a vacuum column number and an enriching column number around.
[0025]C-3. a re-separating operation (1) valve channel -- a by-path pipe -- consider it as the 2nd column of 11-vacuum column 15-1-enriching column 21-1-23-detector 24-outlet 25.
(2) Flow methanol from the pump 3, make 0.01M sodium-dihydrogenphosphate solution (pH=7.5) or 0.01M beta cyclodextrin + sodium acetate solution flow from the pump 5, and the 2nd column 23 re-separates separation the unattained fraction in the enriching column 21-1.
(3) Subsequent operations repeat operation of (1) and (2) except passing an enriching column number around.
[0026]C-4. a result -- the amino acid by which optical resolution was not carried out by said B paragraph -- that is, As a result of preparative isolation, concentration, and the 2nd column re-separating the peak group of threonine, histidine, phenylalanine and ricin, and leucine into a vacuum column, optical resolution was attained as the purpose and the result was shown in drawing 3, drawing 4, and drawing 5. By one analysis condition, the preparative isolation which uses this system also at separation an unattained peak, concentration, and re-separation with the 2nd column proved conventionally that peak division is attained efficiently so that this result might show.

[0027]

[Effect of the Invention]As explained above, according to the device and method of this invention, the non-detachable complicated multicomponent separation is attained by a single analysis condition. Various separation results become available by pouring of the once of a sample, and waste of a precious sample is prevented, and it is also possible to save the time and effort of sample re-preparation.

[Translation done.]

Drawing selection Drawing 1



[Translation done.]